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Live-cell imaging for synaptic vesicle precursors in human iNeuron axons

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We use this protocol and it's working

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Abstract

Here, we describe procedure and equipment used for live-imaging of synaptic vesicle precursors. This was performed using DIV21 human iPSC-derived excitatory glutamatergic neurons. Equipment and software used varied based on scheduled upgrades to microscopy equipment during the course of this study.

Materials

1. GlutaMAX™ SupplementGibco - Thermo FisherCatalog #35050061
2. B-27™ Supplement (50X), serum freeGibco - Thermo FisherCatalog #17504044
3. Hibernate A low fluorescence media (BrainBits, Cat# HALF)
4. Recombinant Human NT-3peprotechCatalog #450-03
5. Recombinant Human/Murine/Rat BDNFpeprotechCatalog #450-02

Safety warnings

Safety information

Investigators should be trained and familiar with the confocal microscope to avoid eye damage from lasers.

1

Note

Please refer to "Protocol: Culture and transfection of iPSC-derived neurons for live-imaging of axonal cargoes" for plating and transfection instructions.

Image human iNeurons on DIV21, 48-72 hours after transfection with PGK-mScarlet-synaptophysin.

2 Replace culture media with low fluorescence imaging media.

2.1 For iNeurons, use Hibernate A medium supplemented with:

	A	B
	BDNF	10 ng/mL
	NT-3	10 ng/mL
	B-27	2%

3 Image using spinning disk confocal microscope under 60x magnification (oil immersion objective). See “Materials and Methods” for specific microscopes and cameras used.

4 Identify axons of transfected neurons based on morphological parameters. (Boecker et al., 2020; Kaech and Banker, 2006). For example, axons can most reliably be identified by their length and should span over at least 500 µm.

5 Identify the neuronal soma and measure ~100-150 µm from the soma. Create an ROI that includes a segment of the axon that is in the same Z plane. Image several frames at this field of view prior to photobleaching. Perform one photobleaching cycle with the 405 nm laser for 3 ms/pixel. Dramatically decreased mScarlet-SYP signal should be observed in the ROI following photobleaching, and entry of mScarlet-SYP+ vesicles into the bleached region should be readily observed.

6 Acquire time lapse recordings at a frame rate of 5 frames per second for 00:05:00 . 5m

Note

- Rapid framerate is preferable due to the high transport speed of SVPs
- Knowledge of the pixel/micron ratio for the specific objective and camera being used is necessary for accurately measuring distances.

Protocol references

1. Boecker, C.A., Olenick, M.A., Gallagher, E.R., Ward, M.E., and Holzbaur, E.L.F. (2020). ToolBox: Live Imaging of intracellular organelle transport in induced pluripotent stem cell-derived neurons. Traffic 21, 138–155.
2. Kaech, S., and Banker, G. (2006). Culturing hippocampal neurons. Nat. Protoc. 1, 2406–2415.